

USE OF 2-METHYLENE-19-NOR-20(S)-1 α ,25-DIHYDROXYVITAMIN D₃
TO INCREASE THE LIFE EXPECTANCY OF HUMAN BEINGS

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BACKGROUND OF THE INVENTION

5 This invention relates to vitamin D compounds, and more particularly to pharmaceutical uses for 2-methylene-19-nor-20(S)-1 α ,25-dihydroxyvitamin D₃.

The natural hormone, 1 α ,25-dihydroxyvitamin D₃ and its analog in ergocalciferol series, i.e. 1 α ,25-dihydroxyvitamin D₂ are known to be highly potent regulators of calcium homeostasis in animals and humans, and more recently their activity in cellular differentiation has been established, Ostrem et al.,
10 Proc. Natl. Acad. Sci. USA, 84, 2610 (1987). Many structural analogs of these metabolites have been prepared and tested, including 1 α -hydroxyvitamin D₃, 1 α -hydroxyvitamin D₂, various side chain homologated vitamins and fluorinated analogs. Some of these compounds exhibit an interesting separation of activities
15 in cell differentiation and calcium regulation. This difference in activity may be useful in the treatment of a variety of diseases as renal osteodystrophy, vitamin D-resistant rickets, osteoporosis, psoriasis, and certain malignancies.

Recently, a relatively new class of vitamin D analogs has been discovered, i.e. the so called 19-nor-vitamin D compounds, which are
20 characterized by the replacement of the A-ring exocyclic methylene group (carbon 19), typical of the vitamin D system, by two hydrogen atoms. Biological testing of such 19-nor-analogs (e.g., 1 α ,25-dihydroxy-19-nor-vitamin D₃) revealed a selective activity profile with high potency in inducing cellular differentiation, and very low calcium mobilizing activity. Thus, these compounds are potentially useful
25 as therapeutic agents for the treatment of malignancies, or the treatment of various skin disorders. Two different methods of synthesis of such 19-nor-vitamin D analogs have been described (Perlman et al., Tetrahedron Lett. 31, 1823

(1990); Perlman et al., *Tetrahedron Lett.* 32, 7663 (1991), and DeLuca et al., U.S. Pat. No. 5,086,191).

In U.S. Pat. No. 4,666,634, 2 β -hydroxy and alkoxy (e.g., ED-71) analogs of 1 α ,25-dihydroxyvitamin D₃ have been described and examined by Chugai group as potential drugs for osteoporosis and as antitumor agents. See also
5 Okano et al., *Biochem. Biophys. Res. Commun.* 163, 1444 (1989). Other 2-substituted (with hydroxyalkyl, e.g., ED-120, and fluoroalkyl groups) A-ring analogs of 1 α ,25-dihydroxyvitamin D₃ have also been prepared and tested (Miyamoto et al., *Chem. Pharm. Bull.* 41, 1111 (1993); Nishii et al., *Osteoporosis*
10 *Int. Suppl.* 1, 190 (1993); Posner et al., *J. Org. Chem.* 59, 7855 (1994), and *J. Org. Chem.* 60, 4617 (1995)).

Recently, 2-substituted analogs of 1 α ,25-dihydroxy-19-nor-vitamin D₃ have also been synthesized, i.e. compounds substituted at 2-position with hydroxy or alkoxy groups (DeLuca et al., U.S. Pat. No. 5,536,713), which exhibit interesting
15 and selective activity profiles. All these studies indicate that binding sites in vitamin D receptors can accommodate different substituents at C-2 in the synthesized vitamin D analogs.

In a continuing effort to explore the 19-nor class of pharmacologically important vitamin D compounds, an analog which is characterized by the presence of a methylene substituent at the carbon 2 (C-2) has been synthesized
20 and tested. Of particular interest is the analog which is characterized by the unnatural configuration of the methyl group at carbon 20 (C-20), i.e. 2-methylene-19-nor-20(S)-1 α ,25-dihydroxyvitamin D₃. This vitamin D analog seemed an interesting target because the relatively small methylene group at C-2 should not
25 interfere with the vitamin D receptor. Moreover, molecular mechanics studies performed on the model 1 α -hydroxy-2-methylene-19-nor-vitamins indicate that such molecular modification does not change substantially the conformation of the cyclohexanediol ring A. However, introduction of the 2-methylene group into

19-nor-vitamin D carbon skeleton changes the character of its 1 α - and 3 β - A-ring hydroxyls. Both hydroxyls are allylic to the exocyclic methylene group similar to the 1 α -hydroxyl group (crucial for biological activity) in the molecule of the natural hormone, 1 α ,25-(OH)₂D₃.

5 2-methylene-19-nor-20(S)-1 α ,25-dihydroxyvitamin D₃ is known, and this compound exhibits a desired, and highly advantageous, pattern of biological activity which has been reported in DeLuca et al U.S. Patent 5,843,928. This compound is characterized by intestinal calcium transport activity, similar to that of 1 α ,25-dihydroxyvitamin D₃, but exhibiting very high activity, as compared to
10 1 α ,25-dihydroxyvitamin D₃, in its ability to mobilize calcium from bone. Hence, this compound is highly specific in its calcemic activity. Its preferential activity on mobilizing calcium from bone allows the in vivo administration of this compound for the treatment of metabolic bone diseases where bone loss is a major concern. Because of its preferential activity on bone, this compound would be a preferred
15 therapeutic agent for the treatment of diseases where bone formation is desired, such as osteoporosis, especially low bone turnover osteoporosis, steroid induced osteoporosis, senile osteoporosis or postmenopausal osteoporosis, as well as osteomalacia and renal osteodystrophy.

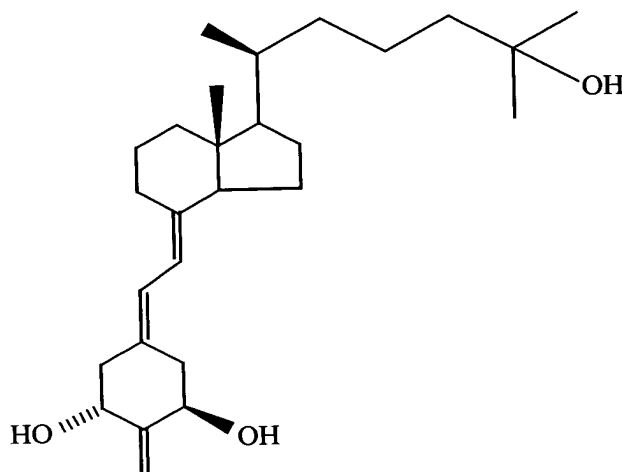
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SUMMARY OF THE INVENTION

The present invention is directed toward various pharmaceutical uses for 2-methylene-19-nor-20(S)-1 α ,25-dihydroxyvitamin D₃. (See formula I and hereinafter referred to as "2-MD") In particular, the present invention demonstrates that administration of 2-MD will substantially increase the life
25 expectancy of human beings, especially elderly human beings. More specifically, 2-MD increases the survival of females lacking estrogen (e.g. post-menopausal females), and reduces mortality resulting from malignant tumors in human beings (both male and female) by inhibiting tumorigenesis in the treatment of a cancer

such as skin cancer, lung cancer, leukemia, colon cancer, breast cancer or prostate cancer.

Structurally this 19-nor analog is characterized by the general formula **I** shown below:



I

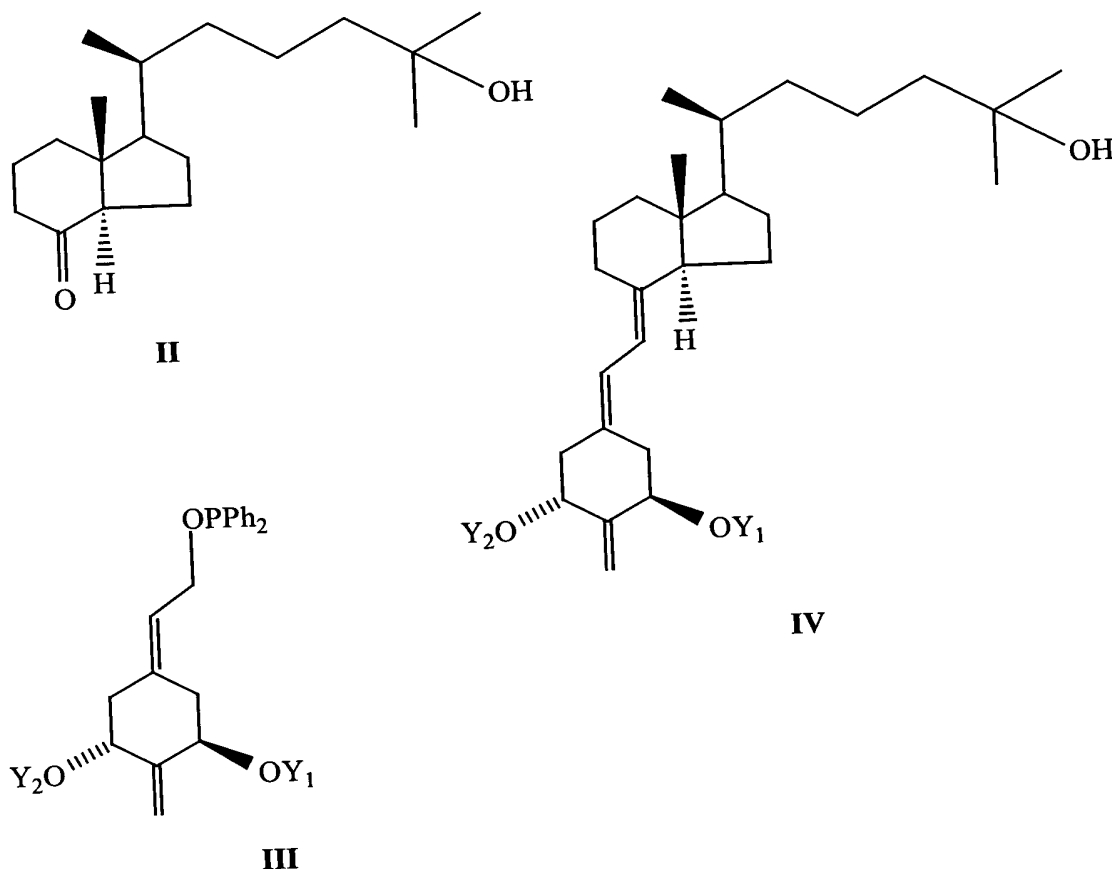
The solid wedge-shaped line to the methyl substituent at C-20 indicates that carbon 20 has the S configuration. The compound may be present in a composition in an amount from about 0.1 $\mu\text{g/gm}$ to about 50 $\mu\text{g/gm}$ of the composition, and may be administered in dosages of from about 0.1 $\mu\text{g/day}$ to about 100 $\mu\text{g/day}$. The treatment may be transdermal, oral or parenteral.

The above compound is characterized by high cell differentiation activity. Thus, this compound also provides a therapeutic agent for the treatment of malignancies, especially as an anti-tumor agent to inhibit tumorigenesis in persons afflicted with skin cancer, lung cancer, leukemia, colon cancer, breast cancer and prostate cancer.

DETAILED DESCRIPTION OF THE INVENTION

2-methylene-19-nor-20(S)-1 α ,25-dihydroxyvitamin D₃ (referred to herein as 2-MD) was synthesized and tested. Structurally, this 19-nor analog is characterized by the general formula **I** previously illustrated herein.

The preparation of 2-methylene-19-nor-20(S)-1 α ,25-dihydroxyvitamin D₃ having the basic structure I can be accomplished by a common general method, i.e. the condensation of a bicyclic Windaus-Grundmann type ketone II with the allylic phosphine oxide III to the corresponding 2-methylene-19-nor-vitamin D analog IV followed by deprotection at C-1 and C-3 in the latter compound:



In the structures II, III, and IV groups Y₁ and Y₂ are hydroxy-protecting groups, it being also understood that any functionalities that might be sensitive, or that interfere with the condensation reaction, be suitably protected as is well-known in the art. The process shown above represents an application of the convergent synthesis concept, which has been applied effectively for the preparation of vitamin D compounds [e.g. Lythgoe et al., J. Chem. Soc. Perkin Trans. I, 590 (1978); Lythgoe, Chem. Soc. Rev. 9, 449 (1983); Toh et al., J. Org. Chem. 48,

1414 (1983); Baggiolini et al., J. Org. Chem. 51, 3098 (1986); Sardina et al., J. Org. Chem. 51, 1264 (1986); J. Org. Chem. 51, 1269 (1986); DeLuca et al., U.S. Pat. No. 5,086,191; DeLuca et al., U.S. Pat. No. 5,536,713].

Hydrindanones of the general structure II are known, or can be prepared
5 by known methods.

For the preparation of the required phosphine oxides of general structure III, a new synthetic route has been developed starting from a methyl quinate derivative which is easily obtained from commercial (1R,3R,4S,5R)-(-)-quinic acid as described by Perlman et al., Tetrahedron Lett. 32, 7663 (1991) and DeLuca et
10 al., U.S. Pat. No. 5,086,191.

The overall process of the synthesis of compound I is illustrated and described more completely in U.S. Patent No. 5,843,928 issued December 1, 1998 and entitled "2-Alkylidene-19-Nor-Vitamin D Compounds" the specification of which is specifically incorporated herein by reference. The biological activity of
15 2-MD is reported in U.S. Patent Application No. 09/616,164, filed July 14, 2000, the specification of which is also specifically incorporated herein by reference.

EXAMPLE 1

This example demonstrates that in female rats that have undergone
20 ovariectomy, their survival is significantly increased if they are given 2-methylene-19-nor-(20S)-1,25-(OH)₂D₃ each day at 5-7 ng/kg/body weight orally. Thus, the experiment was carried out as follows:

48 retired female breeder rats of 12 months of age were obtained from the Harlan Sprague-Dawley Company. Upon arrival, the animals were
25 ovariectomized or sham-operated. These animals were housed in individual hanging wire cages and provided water ad libitum. Food consumption was restricted to 19-21 g/day. Animals were maintained on a purified diet described by Suda et al (J. Nutr. 1970) which contained 0.47% calcium and 0.3%

phosphorus. In addition, these animals received supplements of vitamin A, E, D, and K as described in the Suda paper. One half of the animals received each day 0.1 ml of Wesson Oil while the remaining one-half of the animals received orally the 0.1 ml of Wesson Oil containing 5 or 7 ng/2-MD/kg body weight. The animals were monitored weekly for body weight, general health and survival. These animals were followed for 7.5 months. During the course of this period, 4 of the 24 animals receiving the purified diet without supplementation failed to survive largely due to the development of mammary tumors. On the other hand, all animals receiving 2-MD survived the entire period and were in good health.

These results demonstrate that 2-MD increases the survival rate to 100% in the aged, ovariectomized female rats. The mechanism appears to be inhibition of tumorigenesis inasmuch as at least 3 of the controls who failed to survive had developed tumors while the fourth died of unknown causes. The tabular data in Table 1 demonstrate the survival data and also provide the average body weight initially and at 7-1/2 months.

Table 1
Survival and Body Weight in Aged Female Rats

Treatment	Survival Ratio #premature deaths/total # animals	Body Weight g	
		Initial	Final
Vehicle	4/24	343±6	367±10
2-MD	0/24	361±8	382±10

For treatment purposes, the compound of this invention defined by formula I may be formulated for pharmaceutical applications as a solution in innocuous solvents, or as an emulsion, suspension or dispersion in suitable

solvents or carriers, or as pills, tablets or capsules, together with solid carriers, according to conventional methods known in the art. Any such formulations may also contain other pharmaceutically-acceptable and non-toxic excipients such as stabilizers, anti-oxidants, binders, coloring agents or emulsifying or taste-modifying agents.

5 The compound may be administered orally, topically, parenterally or transdermally. The compound is advantageously administered by injection or by intravenous infusion of suitable sterile solutions, or in the form of liquid or solid doses via the alimentary canal, or in the form of creams, ointments, patches, or
10 similar vehicles suitable for transdermal applications. Doses of from 0.1 μ g to 100 μ g per day of the compounds are appropriate for treatment purposes, such doses being adjusted according to the disease to be treated, its severity and the response of the subject as is well understood in the art. Since the compound exhibits specificity of action, each may be suitably administered alone, or together
15 with graded doses of another active vitamin D compound -- e.g. 1 α -hydroxyvitamin D₂ or D₃, or 1 α ,25-dihydroxyvitamin D₃ -- in situations where different degrees of bone mineral mobilization and calcium transport stimulation is found to be advantageous.

Compositions for use in the above-mentioned treatment of malignancies
20 comprise an effective amount of the 2-methylene-20(S)-19-nor-vitamin D compound as defined by the above formula I as the active ingredient, and a suitable carrier. An effective amount of such compound for use in accordance with this invention is from about 0.01 μ g to about 50 μ g per gm of composition, and may be administered topically, transdermally, orally or parenterally in dosages of
25 from about 0.1 μ g/day to about 100 μ g/day.

The compound may be formulated as creams, lotions, ointments, topical patches, pills, capsules or tablets, or in liquid form as solutions, emulsions, dispersions, or suspensions in pharmaceutically innocuous and acceptable

solvent or oils, and such preparations may contain in addition other pharmaceutically innocuous or beneficial components, such as stabilizers, antioxidants, emulsifiers, coloring agents, binders or taste-modifying agents.

5 The compound is advantageously administered in amounts sufficient to effect the differentiation of promyelocytes to normal macrophages. Dosages as described above are suitable, it being understood that the amounts given are to be adjusted in accordance with the severity of the disease, and the condition and response of the subject as is well understood in the art.

10 The formulations of the present invention comprise an active ingredient in association with a pharmaceutically acceptable carrier therefore and optionally other therapeutic ingredients. The carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulations and not deleterious to the recipient thereof.

15 Formulations of the present invention suitable for oral administration may be in the form of discrete units as capsules, sachets, tablets or lozenges, each containing a predetermined amount of the active ingredient; in the form of a powder or granules; in the form of a solution or a suspension in an aqueous liquid or non-aqueous liquid; or in the form of an oil-in-water emulsion or a water-in-oil emulsion.

20 Formulations for rectal administration may be in the form of a suppository incorporating the active ingredient and carrier such as cocoa butter, or in the form of an enema.

Formulations suitable for parenteral administration conveniently comprise a sterile oily or aqueous preparation of the active ingredient which is preferably isotonic with the blood of the recipient.

25 Formulations suitable for topical administration include liquid or semi-liquid preparations such as liniments, lotions, applicants, oil-in-water or water-in-

oil emulsions such as creams, ointments or pastes; or solutions or suspensions such as drops; or as sprays.

For asthma treatment, inhalation of powder, self-propelling or spray formulations, dispensed with a spray can, a nebulizer or an atomizer can be used.

- 5 The formulations, when dispensed, preferably have a particle size in the range of 10 to 100 μ .

- The formulations may conveniently be presented in dosage unit form and may be prepared by any of the methods well known in the art of pharmacy. By the term "dosage unit" is meant a unitary, i.e. a single dose which is capable of
- 10 being administered to a patient as a physically and chemically stable unit dose comprising either the active ingredient as such or a mixture of it with solid or liquid pharmaceutical diluents or carriers.